ABSTRACT

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Tested reactivators are substances with oxime moiety attached to a ring with quaternary nitrogen, which enable binding to active site of acetylcholinesterase (AChE). This nucleophilic structure can reactivate inhibited AChE by organophosporus compounds. Reactivation is possible only if the bond between inhibitor and AChE is not established (Kassa, 2002). Reactivators have relative high affinity to active site of AChE. The value of affinity can be expressed by their values of inhibition potential.

The aim of this study was observed influence of structural changes in oximes to AChE inhibition ability. This knowledge can be used in further designing new structures with peripheral and reversible activity against to AChE. The mechanism of peripheral inhibition against to AChE is already used in prophylaxis of nerve agent poisoning. The reversible inhibitor binds into AChE active site and thus, prevents subsequent binding of nerve agent. Reversible AChE inhibitors with peripheral effect are also used in Myasthenia gravis treatment.

The reactivator inhibition potency against to AChE was determined by using modified method according by Ellman et al. (1961). It was in vitro testing, the pure recombinant human AChE (rhAChE) was used. The absorbance was 412 nm. The measurement was started in cuvette by addition reactivator (every point in the concentration range from 10^{-1} to 10^{-8} mmol / l) to rhAChE. The inhibition time period was 10 min, after this time interval phosphate buffer (0.1 M, pH 7.4) with DTNB and solution with acetylthiocholine was added. This measuring of each concentration points were triplicate. The blind sample was also tested for each concentration range; it shows activity of enzyme

without influence of reactivator. Then was measured oximolysis to avoid reading false-positive results, because reactivators in higher concentrations are able to split DTNB.

Structural influence on inhibition potency was studied on tested reactivators with different constitutions. It was determined that longer connecting chain in bisquaternary oxime was able to increased inhibition potency against AChE. Meta position in monoquaternary and ortho position in bisquaternary oximes also increase inhibition potential. Next structural sign that increase inhibition potency was substitution in connecting chain. However, double bond in linker decreased inhibition effect.